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Postnatal Supplemental Choline Facilitates Extinction of Fear in Adolescent Rats

Natasha Chandini Hakkal
College of William and Mary

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Natasha Chandini Hakkal

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(Honors)

Pamela Hunt, Director

Randolph Coleman
Randolph Coleman

Christine Niehaus
Christine Niehaus

Williamsburg, VA
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Abstract

The effects of supplemental choline administration on the acquisition of trace and long-delay conditioning was examined in Sprague–Dawley rats. The first experiment documented the acquisition of fear in adolescent rats using several different conditioning procedures. In the second experiment, choline was administered on postnatal days 15-26. The subjects were then trained on postnatal day 30 using either trace or long-delay conditioning procedures. It was expected that the choline would improve hippocampus projections and facilitate the acquisition of trace conditioning. However, the results of this experiment showed that the choline had no effect on acquisition of responding, but instead promoted extinction in both the long-delay and trace conditioned rats. This effect is possibly due to the supplemental choline casuing improvements of the prefrontal cortex, a region involved in extinction.

Introduction

Research into the function of choline is still in the early stages. Choline is known to be necessary for proper development and function of the nervous system and may even afford protective effects against age-related cognitive decline and exposure to toxins. Choline is an essential nutrient, is an organic compound found in the lipids of cell membranes, and is the precursor to the neurotransmitter acetycholine (ACh). Choline is necessary for synthesis of the phospholipids of cell membranes, cholinergic neurotransmission, transmembrane signaling, and lipid-cholesterol transport and metabolism (Zeisel, 2000). It helps to prevent fat and cholesterol accumulation in the liver. Choline is also necessary for the growth of mammalian cells. Most choline is found in phospholipids such as sphingomyelin and phosphatidylcholine, which are the major membrane components of all cells including glia and neurons. Although the body can produce low amounts of choline, a healthy diet consists of choline-rich foods such as eggs or whole grains. In animal models, choline provided to pregnant or lactating females is transferred to the fetus via placenta or milk. Choline causes changes in the dam's metabolism and has multiple other effects in the offspring (Montoya et al., 2000).

As a precursor to acetylcholine, choline is necessary for synaptic transmission in cholinergic pathways in the brain, including those projecting to the hippocampus. Hippocampal neurons utilize acetylcholine when forming memories (e.g. Anagnostaras, Maren, Sage, Goodrich & Fanselow, 1999; Tinsley, Quinn & Fanselow, 2004). This evidence comes from several sources, including studies of patients with Alzheimer's disease. The level of acetylcholine in an Alzheimer's patient is severely diminished and

the hippocampus is atrophied, which correlates with memory loss (cf. Moore & Claflin, 2008). The availability of choline during the second half of fetal development alters the development of the hippocampal cholinergic system. Supplemental dietary choline during pre- and/or early postnatal life has been shown to produce long-lasting improvements in many aspects of cognition.

Meck, Williams, and their colleagues have reported that rats given extra dietary choline prenatally performed better in hippocampus- and cholinergic-dependent tasks as adults. (Cheng & Meck, 2007; Meck & Williams, 1997, 1999, 2003; Meck, Smith & Williams, 1988, 1989). Rats in these studies showed improvement in spatial memory tasks, greater temporal discrimination abilities, and exhibited a significant reduction in proactive interference. It was also found that prenatal choline supplementation resulted in a sustained depolarization during induction of long-term-potentiation (LTP), therefore lowering the threshold for LTP induction, in area CA1 of the hippocampus (Montoya et al., 2000). LTP improves the communication between a presynaptic and postsynaptic neuron by enhancing synaptic transmission. It is an important cellular mechanism for some types of learning and memory. The CA1 area of the hippocampus is necessary for forming spatial memories in rats. Therefore, increasing synaptic efficacy in this area was proposed as the basis for improved performance in spatial memory tasks. By lowering the threshold of LTP induction, choline may have promoted the formation of spatial memories, leading to the improvement in performance demonstrated by choline-treated rats.

Supplemental choline administration has been shown to result in substantive changes in cholinergic and hippocampal anatomy and function (Jones, Meck, Williams,

Wilson & Swartzwelder, 1999; Montoya et al., 2000). Choline availability during sensitive pre-and postnatal periods in rats (E12-17, PD 16-30) is essential for the proper development of the hippocampus. Early choline supplementation leads to long-term improvement in the efficiency of the cholinergic system (Meck et al., 1989). However, the effects of choline on neurochemical mechanisms that lead to improvement in memory are unknown. It is likely that additional choline affects ACh synthesis and release, the basal forebrain cholinergic neurons involved in memory processes, and/or the choline-containing phospholipids in cell signaling events in the brain (Cermak, Holler, Jackson & Blusztajn, 1998). Prenatal choline supplementation increased basal and glutamate receptor-stimulated phospholipase-D activity in the hippocampus of adult offspring (Montoya et al., 2000). Additionally, it decreased acetylcholinesterase (AChE) activity in the hippocampus of juvenile offspring. AChE is the enzyme that breaks down acetylcholine. Because ACh is inactivated only through enzymatic degradation processes, reductions in levels of AChE lead to longer duration of action of ACh in the synaptic cleft. The cholinergic system of the hippocampus is organized so that it is very efficient at recycling and reusing choline for acetycholine synthesis. Choline supplemented rats have reduced AChE activity because there is more choline available, resulting in enhanced cholinergic neurotransmission (Cermak et al., 1998). Furthermore, choline supplementation may also facilitate the birth, death, and migration of cells in the hippocampus during fetal brain development, thereby altering the neurons involved in memory storage in the brain (Zeisel, 2000). Choline has been the focus of numerous studies examining the effects of pre-, peri-, and postnatal supplementation on cognitive

development in rats. Researchers have specifically studied the effects of choline on learning tasks that require hippocampal activity.

Early choline administration was reported to facilitate the developmental emergence of spatial memory using the Morris water maze task (Meck & Williams, 2003). Young rats typically do not exhibit spatial memory until about postnatal day 25 (post-weaning; Rudy, Stadler-Morris & Albert, 1987). It is at about this age that LTP in hippocampal slices can first be observed (Rudy, 1991), and the relatively late development of spatial memory abilities has been linked to the late functional development of the hippocampus (Rudy, 1991). In addition to spatial memory, the hippocampus has been implicated in many other learning and memory phenomena. Included in this list are several types of Pavlovian conditioning. Contextual fear conditioning is one example. Context refers to the general environment in which learning takes place, and the ability to learn about the context is severely attenuated in animals with hippocampal lesions. Context conditioning is also relatively late to emerge ontogenetically (Rudy & Morledge, 1994), being apparent no earlier than about postnatal day 23. There are no reports of the effects of early choline supplementation on the development of contextual learning abilities, although it is likely that context conditioning results would parallel those of the effects of supplemental choline on spatial memory abilities.

Another type of hippocampus-dependent Pavlovian conditioning that has becoming a valuable tool for studying hippocampal function is known as trace conditioning. In Pavlovian fear conditioning, a conditioned stimulus (CS), such as a tone or a light, is paired with an unconditioned stimulus (US), usually a mild footshock. In

delay conditioning, the CS and US overlap and coterminate, ending the trial. In *trace* conditioning, the CS is presented, but the US is not presented until after a stimulus-free period, known as the trace interval. While rats as young as 17 days of age can exhibit delay conditioning, acquisition of trace conditioning is not evident until about 25 days of age (Barnet & Hunt, 2005; Moye & Rudy, 1987). In trace conditioning, rats must hold a memory of the CS during the trace interval, a process that requires the hippocampus (McEchron, Tseng & Disterhoft, 2003; Woodruff-Pak & Disterhoft, 2008). Trace conditioning differs from delay conditioning in two important ways. First, the trace procedure includes a stimulus-free period between the CS offset and US onset. Second, the interstimulus interval (ISI), which is defined as the time from onset of the CS until onset of the US, is longer in the trace procedure than in the delay procedure (see Figure 1). To control for this second difference, *long-delay* conditioning is used. Long-delay procedures use the same CS-US relationship as delay conditioning, but the ISI is increased to match that of the trace conditioning procedure. With a visual CS, acquisition of long-delay and trace conditioning emerge at about the same time in rats (Barnet & Hunt, 2005; Hunt & Richardson, 2007). However, long-delay conditioning is not thought to be hippocampal dependent. Long-delay conditioning may emerge later in rats because of other required neural systems that develop in tandem with the hippocampus.

Recently, Moore and Claflin (2008) reported that supplemental choline administration to young rats on postnatal days (PD) 15-26 facilitated later acquisition of trace eyeblink conditioning. Eyeblink conditioning involves cerebellar circuits (Kim & Thompson, 1997), and trace eyeblink conditioning additionally requires the hippocampus (Moyer, Deyo & Disterhoft, 1990). Moore and Claflin (2008) reported that supplemental

choline resulted in faster acquisition of trace eyeblink conditioning, but this treatment has no effect on long-delay eyeblink conditioning. These data demonstrates that choline promotes earlier development of hippocampal, and perhaps cholinergic, systems that are critical for trace but not delay learning.

The purpose of the present experiments was to determine whether administration of choline during the postnatal period would result in improved learning in a trace fear-conditioning task in animals tested as adolescents. In Experiment 1, we first examined the acquisition of fear in experimentally-naïve adolescent rats using different conditioning procedures. On the basis of the data obtained, Experiment 2 examined the effects of supplemental choline on trace and long-delay conditioning. We predicted that extra dietary choline given during the early postnatal period would facilitate the acquisition of trace fear conditioning but not the acquisition of long-delay fear conditioning (cf. Moore & Claflin, 2008).

Experiment 1

The purpose of Experiment 1 was to document acquisition of fear in adolescent rats using several different conditioning procedures including delay, trace, and long delay. As stated previously, in delay conditioning the CS is presented and is overlapped by the presentation of the US whereas during trace conditioning the CS and US do not overlap. In trace conditioning, the CS is presented and is followed by a period of time in which no stimulus is presented. This is known as a stimulus free trace interval. Furthermore, the time from onset of the CS to onset of the US is referred to as the inter-stimulus interval (ISI). The ISI is necessarily longer in the trace procedure than the delay procedure (see Figure 1). In order to assess the effects of the longer ISI, researchers created a training procedure called long delay conditioning, in which the CS is presented and is overlapped by the presentation of the US as it is in delay conditioning. However, the period of time between when the CS is presented and when the US is presented is increased to match that of the trace conditioning procedure. For example, if the ISI for delay conditioning were 10 seconds, the ISI for trace and long delay conditioning would be 20 seconds.

Previous experiments have shown that trace and long-delay fear conditioning to a visual CS emerge at approximately the same age, on postnatal day 25 (Barnet & Hunt, 2005; Moye & Rudy, 1987), whereas delay conditioning is evident several days earlier in development, on about postnatal day 18. This suggests that the critical difference in learning between delay and trace conditioning exists because of the longer ISI rather than just the unfilled gap between the CS and US. It has been suggested that trace conditioning

does not emerge until 25 days of age due to hippocampal immaturity (Moye & Rudy, 1987). However, it is unclear why long delay conditioning is also developmentally delayed, as this training procedure does not require the hippocampus. Pharmacological manipulations of the hippocampus (Hunt & Richardson, 2007) or electrolytic lesions to this region (Ivkovich & Stanton, 2001) severely impair trace conditioning in both fear and eyeblink preparations, but have no obvious effect on long delay learning. It has been suggested that neural systems outside the hippocampus that develop in tandem with the hippocampus mediate long-delay learning.

In the present experiment, the animals were assigned to one of four groups: delay, trace, long delay, or unpaired CS–US pairings. In unpaired conditioning, the CS and US are not presented together. The computer program randomly generated a US once during the CS-CS interval with the stipulation that a US could not occur 60 seconds prior to, or following, a CS. Subjects were tested for CS-elicited freezing 24 h after training. Freezing was defined as the absence of observable movements except those necessary for respiration (Fanselow, 1980). The prediction was that subjects in the delay group would exhibit the most freezing, subjects in the unpaired group would exhibit the least amount of freezing, and subjects in the long delay and trace groups would exhibit intermediate and comparable amounts of freezing. These predictions were based on data reported by Barnet and Hunt (2005).

Method

Subjects. Fifty 30-day-old Sprague–Dawley-derived rats representing 5 litters were randomly assigned to one of four groups, delay, trace, long delay, or unpaired (ns =

10/group). The subjects were born and reared in the vivarium at the College of William and Mary. Males and females used for breeders were obtained from Charles River Laboratories (Wilmington, MA) and were housed in pairs in 50.8 × 40.6 × 21.6 cm polycarbonate cages with wire lids and pine chip bedding. Food (LabDiet Formula 5008) and water were available ad libitum. Cages were checked daily for pups and the day of birth was designated as postnatal day (PD) 0. Litters were culled to 10 pups on PD 2. Pups were weaned on PD 21 and then maintained in a cage with their siblings throughout the experiment. The vivarium was maintained on a 14:10 h light:dark cycle with light onset at 0600 h, and all training and testing procedures occurred during the light portion of the cycle. Procedures were approved by the Institutional Animal Care and Use Committee at the College of William and Mary and conformed to the guidelines established by the National Institutes of Health (1996).

Apparatus. The conditioning trials occurred in two identical modified Skinner boxes, each measuring 38.0 × 26.0 × 22.0 cm. The two shorter walls were made of aluminum and the two longer walls and top were made of Plexiglas. The floor was constructed of 5-mm stainless-steel bars spaced 1.5 cm apart (center-to-center). The grid floor was connected to a custom made constant current shock generator that delivered the 0.5 mA 1 s shock US. Each chamber was located in a custom-built sound-attenuating shell measuring 67.0 × 71.5 × 71.0 cm. A 7-W white light was mounted on an inner wall of the sound-attenuating shell to provide constant low-level illumination. The visual CS was produced by a 25-W white bulb, the center of which was located 12 cm above the floor and 8.5 cm from the rear of the training chamber. The CS flashed at a rate of 2/s in order

prevent acclimatization. All stimulus presentations were controlled by a PC that interfaced Coulbourn Instruments (Allentown, PA) software and hardware.

Testing occurred in one of two novel contexts that were similar except for the dimensions of the sound-attenuating shells. For both, a 29.0 × 21.5 × 46.5 cm clear Plexiglas chamber with an open top and bottom rested on a Plexiglas floor covered with brown paper. The lower 11 cm of the chambers were constructed of horizontally-mounted stainless-steel rods, 5 mm diameter and spaced 1.5 cm apart (center-to-center). The Plexiglas chamber was housed in a sound-attenuating shell (IAC; Industrial Acoustics, New York, NY) with inner walls that were painted black. A 7-W white light was mounted on an inner wall of each IAC to provide constant low-level illumination. Behavior during the test session was videotaped using Sony video cameras (Model CCD-TRV67).

Procedure. On PD 30, the animals were weighed and trained. Animals were placed into the conditioning chamber for an initial 5 min period of adaptation. This was followed by five CS-US pairings. For training, all animals were given 5 presentations each of a flashing 25-W white light (conditioned stimulus; CS) and 1 s, 0.5 mA footshock (unconditioned stimulus; US). The intertribal intervals used for training ranged from 200 to 300 seconds. For delay groups the CS was 10 s in duration and offset of the CS coincided with onset of the US. For long-delay groups the CS duration was 20 s, and the shock was given at the end of the CS. Subjects in the trace group were given trials in which the trace interval separating offset of a 10 s CS from onset of the US was 10 s. Finally, the unpaired control group was given 5 presentations of a 10 s CS and 5

presentations of the US, but in an explicitly unpaired fashion. Animals were removed from the chamber 3 min after the final shock and returned to the home cage. The single training session lasted 30 min.

Testing occurred approximately 24 h after training, on PD 31. Animals were placed into a novel test chamber and given 5 min of adaptation. Subjects were given 5 non-reinforced presentations of the 10 s light CS. The intertribal intervals used for testing ranged from 60 to 120 seconds. The test sessions were videotaped and tapes were later scored by an observer blind to subject group, using a time-sampling procedure. For 10 s prior to each stimulus and during the 10 s of the CS the subject was briefly observed once every 2 s for the presence/absence of freezing. Freezing was defined as the absence of observable movements except those necessary for respiration (Fanselow, 1980).

Statistical analyses. The freezing data were converted into a percentage of intervals scored as freezing (ranging from 0% to 100%) during the pre-CS and CS periods. To assess whether there were group differences in levels of pre-CS freezing the data recorded during the 10 s prior to CS presentation on each of the five test trials were analyzed using mixed-factor Analyses of Variance (ANOVA). The freezing scores during the CS were analyzed similarly.

Results and Discussion

The data obtained from the 40 rats were analyzed. Pre-CS freezing scores were analyzed using a 4 (conditioning treatment) x 5 (test trial) mixed-factor ANOVA. There were no group differences in pre-CS freezing during the test session, $F < 1$. Overall, pre-

CS freezing was relatively low (M = 20.0 +/- 11.4%) and did not change across the test session. There was no effect of testing chamber on levels of freezing [F (1, 38) = 0]. Although the two testing chambers were not identical, differences did not affect responding in animals.

In contrast, the 4 x 5 ANOVA conducted on CS freezing scores revealed a main effect of conditioning, F (3, 36) = 4.22, $p < .05$. Post hoc comparisons made using Newman-Keuls tests ($p < .05$) indicated that the subjects in the delay-conditioning group exhibited the highest levels of freezing. The freezing response of the long delay and trace groups was comparable, but less than that of delay. Subjects in the unpaired group exhibited the lowest levels of freezing. These results are depicted in Figure 2. The freezing scores obtained for these different training groups are comparable to those obtained previously (Barnet & Hunt, 2005). Moreover, there was virtually no extinction, erasing of a behavior, of the freezing response across the test session in any group, as revealed by the lack of significant effects or interactions involving the factor Test Trial.

Experiment 2

The purpose of Experiment 2 was to determine whether supplemental choline administration would facilitate trace fear conditioning, as has been shown for trace eyeblink conditioning (Moore & Claflin, 2008). In Experiment 1, the rats in the trace and long delay groups demonstrated equal amount of freezing, which implies equivalent learning in the two groups, and therefore these two groups were used in the present experiment. The prediction was that choline would facilitate trace conditioning, which depends on the hippocampus, but not long delay conditioning, which does not rely on the hippocampus.

Method

Subjects. Forty rats from four litters, derived from the same source as Experiment 1, were used (ns = 10/group).

Apparatus. The apparatus was the same as used in Experiment 1. Drug injections were given using 1 ml disposable syringes fitted with 30-gauge needles.

Procedure. The design of the experiment was a 2 (drug) x 2 (conditioning treatment) factorial. On postnatal day 15 animals were removed from the home cage as a group and were randomly assigned to one of four groups, designated according to drug administration (saline or choline) and fear conditioning (trace or long delay). Half of the animals were assigned to receive saline and the other half to receive choline. Subjects

were weighed and received one subcutaneous injection of saline or choline chloride (0.10 ml of an 18.8 mg/ml solution of choline chloride dissolved in saline; Meck & Williams, 2003). Immediately after injections all animals were returned to the home cage. Injections continued daily through PD 26. On the last day the subjects were weighed for a final time. On PD 30 animals were trained with trace or long-delay procedures, as described in Experiment 1. Animals were tested approximately 24 h later in a novel context and videotaped records of the test sessions were later scored for CS-elicited freezing.

Results and Discussion

Body weights recorded on the first and last injections days (PD 15 and 26) were analyzed using a 2 (drug) x 2 (sex) x 2 (day) mixed-factor ANOVA. This analysis yielded significant main effects of sex [$F\ (1, 36) = 16.83, p < .01$] and day [$F\ (1, 36) = 2008.00, p < .01$] as well as a Sex x Day interaction [$F\ (1, 36) = 13.33, p < .01$]. Body weights increased from day 15 to day 26, and males gained more weight than females. However, there was no effect or interaction involving the factor Drug, indicating that choline administration did not affect growth of the pups. Body weights are depicted in Table 1. Although the boxes were different sizes, there was no effect of testing chamber on levels of freezing or rate of extinction [$F(1,38)=0.65$].

Levels of pre-CS freezing were analyzed using a 2 (drug) x 2 (conditioning treatment) x 5 (test trial) mixed-factor ANOVA. The analysis revealed a main effect of conditioning treatment, $F\ (1, 36) = 4.18, p < .05$. There were no effects of drug or test

trial. The long delay groups exhibited slightly higher levels of pre-CS freezing during the test (M = 18.8 +/- 7.4%) than trace groups (M = 9.2 +/- 4.5%).

The analysis of CS freezing revealed a main effect of conditioning treatment [$F(1, 36) = 11.29, p < .05$] as well as a Drug x Test Trial interaction, $F(1, 32) = 6.20, p < .05$. Long delay groups showed slightly higher levels of freezing to the CS during the test, which is commensurate with their overall higher levels of pre-CS freezing. The Drug x Test Trial interaction was followed up with analyses comparing the levels of CS freezing exhibited by the groups on Test Trials 1 and 5. As can be seen in Figure 3, saline-treated subjects showed no decrement in responding during the test session, replicating the results of Experiment 1. However, supplemental choline unexpectedly facilitated extinction of acquired fear following both trace and long-delay training. Responding on the last test trial was significantly lower than responding on the first test trial for choline-treated subjects but not for saline-treated subjects. In contrast to our prediction, choline administration had no observable effect on acquisition of trace or long-delay fear conditioning, as evident by equivalent levels of freezing on test trial 1 in saline- and choline-treated subjects.

The data from Experiment 2 failed to show evidence for a facilitation of trace fear conditioning in choline-treated rats, as has been observed for trace eyeblink conditioning (Moore & Claflin, 2008). It is unclear why choline did not have the expected effect in fear conditioning. The neural circuits necessary for delay fear and delay eyeblink conditioning are separable, however. Fear conditioning requires the amygdala (LeDoux, 1995), whereas eyeblink conditioning requires the cerebellum (Kim & Thompson, 1997). The hippocampus, however, has been implicated in trace conditioning in both procedures

(trace fear conditioning: Chowdhury, Quinn & Fanselow, 2005; trace eyeblink conditioning: Moyer, Deyo & Disterhoft, 1990). It is possible that the supplemental choline administration in the Moore and Claflin (2008) study was facilitating the establishment of connections between the hippocampus and cerebellum that underlie trace eyeblink conditioning. Trace fear conditioning involves connections between the hippocampus and amygdala, and the present data tentatively suggest that these neural circuits are not strengthened by choline administration during the period of PD 15-26. Perhaps choline given earlier in development would promote stronger trace fear conditioning. This prediction seems likely because the amygdala develops much earlier than the cerebellum and it is possible that hippocampal connections with these structures are also developmentally dissociated.

Data from Experiment 2 do, however, demonstrate that extra dietary choline can generally facilitate the extinction of fear. Extinction of acquired fear seems to depend on prefrontal cortical systems that interact with the amygdala (Corcoran & Quirk, 2007) and thus supplemental choline may result in long-lasting improvements of frontal cortex function.

General Discussion

These experiments were designed to examine the potential beneficial effects of early supplemental choline administration on the development of a hippocampus-dependent trace fear conditioning task. Experiment 1 was intended to determine the relative strength of conditioning in adolescent rats given delay, trace, or long delay CS-US pairings. On the basis of the data obtained, Experiment 2 examined the effects of early choline administration on performance of subjects in trace and long delay fear conditioning. These two conditioning groups were chosen because, from Experiment 1 results, these two forms of learning were acquired equally by naïve subjects. Experiment 2 was intended as an examination of the effects of choline on the hippocampus, which is required for trace fear conditioning.

It had been previously observed that post-natal injections of choline produced greater learning in trace eyeblink conditioning than in long-delay conditioning (Moore & Claflin, 2008). These results were probably due to the effects of choline on the hippocampus, which is required for trace conditioning but not for long-delay conditioning. Based on Moore and Claflin's experiments, it was expected in the present experiments that choline would facilitate trace fear conditioning but not long-delay fear conditioning. The results of these experiments indicate that while choline fails to facilitate trace fear conditioning, it facilitates extinction of fear following both long-delay and trace conditioning in young rats 30 days of age. Extinction occurs in conditioning when a conditioned response decreases or disappears. This happens when the conditioned stimulus is no longer paired with the unconditioned stimulus. For example, in the present

experiment, extinction occurred when the rats no longer exhibited freezing during the presentation of the light.

The subjects demonstrated extinction after the acquisition of trace fear conditioning and long delay fear conditioning. Extinction occurred during testing when the light CS was presented repeatedly without the shock. The choline subjects froze significantly less on the last test trial compared to the first test trial, whereas the saline-treated subjects exhibited the same amount of freezing throughout the test trials. This latter finding replicated the results obtained in Experiment 1, in which there was no decrement in the freezing response in any of the conditioned groups.

It has been determined that extinction does not require erasing the memory for a conditioned response. Instead, rats create a new memory to suppress the conditioned response (Quirk, Garcia & González-Lima, 2006). Therefore, one or more of the other brain structures must be activated in order to inhibit the old response. The amygdala is critical for the acquisition of fear conditioning. Specifically, the lateral amygdala receives input from the thalamus and other cortical structures about conditioned and unconditioned stimuli (Anglada-Figueroa & Quirk, 2005). The lateral amygdala then projects to the hypothalamus and brain stem to produce the conditioned fear responses. The lateral amygdala also projects both directly and indirectly to the basal amygdala (BA). The BA then projects to the infralimbic prefrontal cortex, a structure implicated in consolidation and storage of extinction memory based on studies showing that destroying the infralimbic prefrontal cortex blocks recall of fear extinction (Anglada-Figueroa & Quirk, 2005). There are also projections from the infralimbic prefrontal cortex back to the amygdala (Quirk et al 2006). Inhibiting these projections could potentially override fear

responses of the amygdala. It has been shown that stimulating the prefrontal cortex strengthens extinction learning (Quirk et al., 2006). Extra dietary choline may induce greater extinction by strengthening the connection between the amygdala and the infralimbic prefrontal cortex and therefore decreasing the conditioned response. Consequently, this supplemental choline may result in long-lasting improvements of the frontal cortex. Further studies could produce information concerning other types of conditioning and extinction to determine if choline only effects fear conditioning.

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Table 1. Mean (+/- SEM) body weights (g) recorded on the first (PD 15) and last (PD 26) day of drug administration. Pups were given a single subcutaneous injection of saline or choline chloride on PD 15-26. Weights are listed separately for male and female pups.

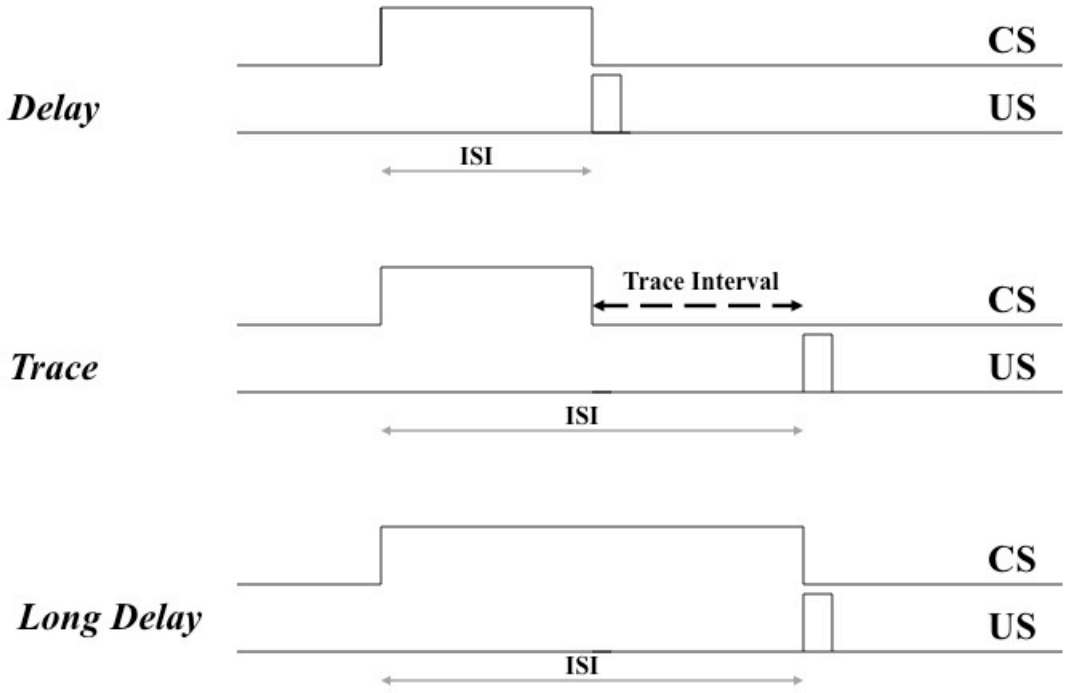
	DRUG			
	Saline		*Choline*	
	Male	Female	Male	Female
AGE				
PD 15	40.2 (1.2)	39.6 (1.7)	42.7 (1.7)	36.4 (1.6)
PD 26	87.4 (2.2)	78.9 (2.0)	89.8 (3.4)	77.2 (2.2)

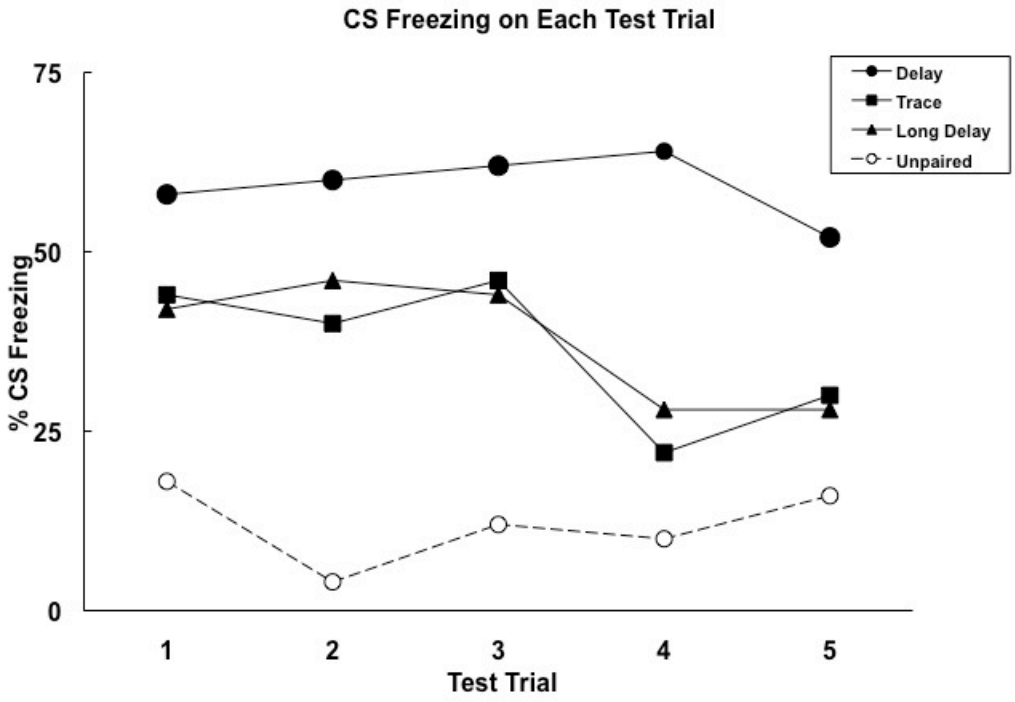
FIGURE CAPTIONS

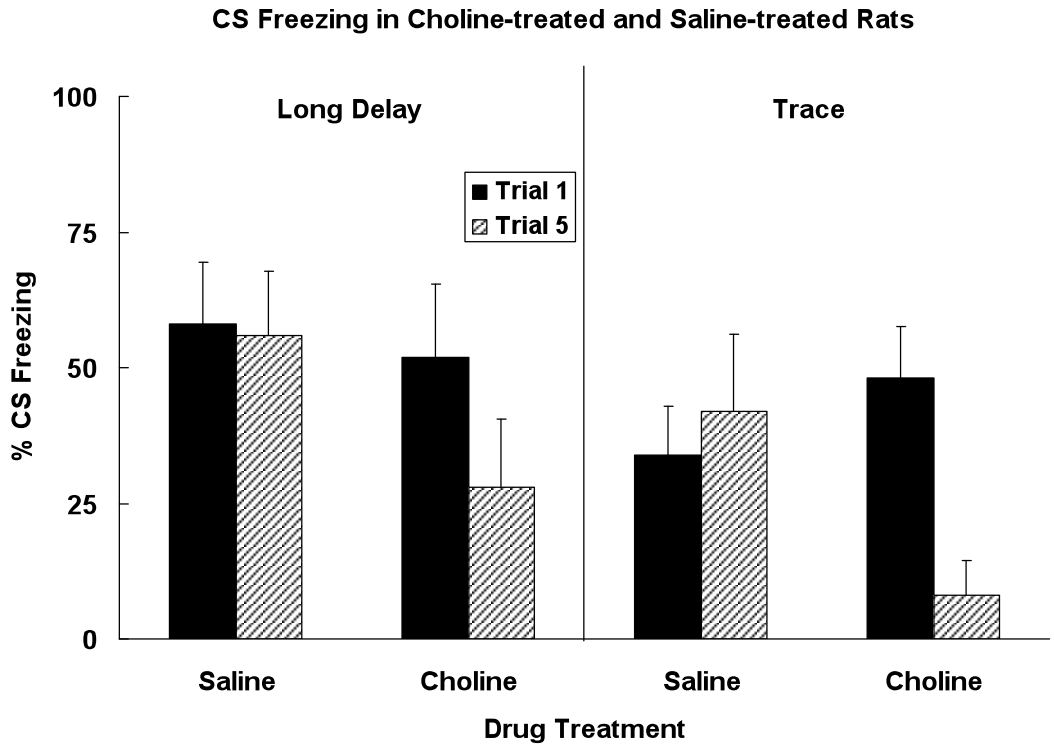
Figure 1. Comparison of the interstimulus interval (ISI) of Delay, Trace, and Long Delay conditioning. The trace procedure includes a stimulus-free period between the CS offset and US onset. The interstimulus interval (ISI) is equal in Trace conditioning and Long-Delay conditioning, but it is shorter in Delay conditioning.

Figure 2. Mean (+/- SEM) % CS freezing recorded during the test session. Untreated subjects were trained with Delay, Trace, Long Delay, or Unpaired on PD 30 and tested 24 h later.

Figure 3. Mean (+/- SEM) % CS freezing recorded during the test session. Animals were administered one subcutaneous injection of saline or choline chloride (0.10 ml of an 18.8 mg/ml solution of choline chloride dissolved in saline) on postnatal days (PD) 15-26. Subjects were trained with either Trace or Long Delay on PD 30 and tested 24 h later.


Delay
CS
US
ISI
Trace
Trace Interval
CS
US
ISI
Long Delay
CS
US
ISI


CS Freezing on Each Test Trial
Delay
Trace
Long Delay
Unpaired
75
50
25
0
% CS Freezing
1
2
3
4
5
Test Trial


CS Freezing in Choline-treated and Saline-treated Rats
Long Delay
Trace
Trial 1
Trial 5
% CS Freezing
100
75
50
25
0
Saline
Choline
Saline
Choline
Drug Treatment